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# Fragile X and other trinucleotide repeat diseases

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## Hereditary unstable DNA

According to the laws of Mendelian genetics, genes are passed unchanged from parent to progeny. New gene mutations can occur, but once they do, the mutations are also passed on unchanged. Although this concept still applies to many genes or traits, it is now recognized that certain genes are inherently unstable, and their size and function may be altered as they are transmitted from parent to child. These intergenerational genetic changes explain such puzzling genetic phenomena as anticipation and skipped generations, and are responsible for several important diseases; at least 20 diseases caused by hereditary unstable DNA have been identified.

Hereditary unstable DNA is composed of strings of trinucleotide repeats. Trinucleotide repeats are stretches of DNA in which three nucleotides are repeated over and over (i.e., CAGCAGCAGCAG). Triplet repeats composed of all combinations of nucleotides have been identified, but CGG and CAG are the most common [1]. These repeats are found in several sites within genes: in the noncoding region, in introns (gene segments that are translated into RNA but are then excised before the mRNA is translated into a protein), or in exons (gene segments that are translated into mRNA and are not excised). Triplet repeats found within exons may be in the untranslated region, or in the region that is translated into protein (Fig. 1) [2].

Depending on their location within the gene, the number of triplet repeats in a string can change as it is passed on to offspring. Although decreases in the number of repeats can occur, the number usually increases. Once the number of repeats reaches a critical size, it can have a variety of effects on gene function. The repeats may cause a loss of gene function, as in fragile X. However, in the majority of triplet diseases the result is the gain of a new, abnormal protein and thus a new function. For example, if the triplet repeat is composed of CAGs, (which encode glutamine), and is located in a coding region, the translated

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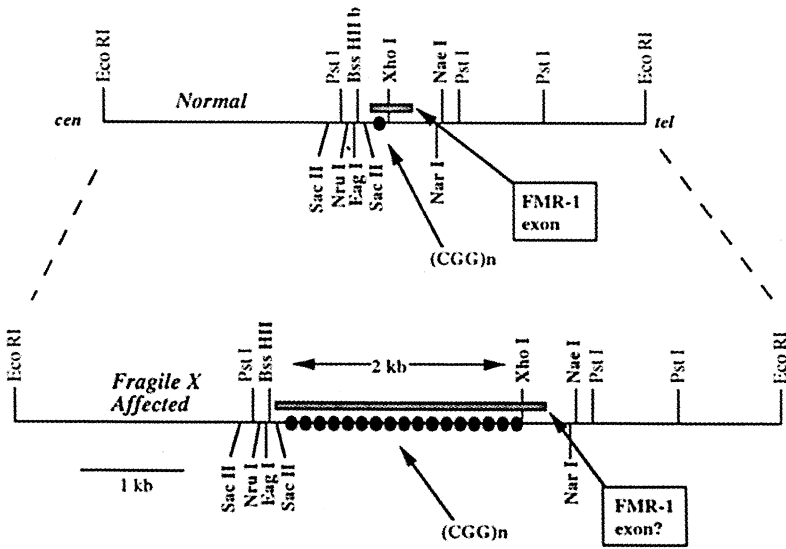


Fig. 1. Diagram of the presumed structure of the FMR 1 gene. Map of the 5.2-kb fragment in Xq27.3 produced by digestion with restriction enzyme *EcoRI*. The fragment contains the CGG repeats (●) mutated in fragile X syndrome in normal and fragile X-affected forms. Restriction sites for other enzymes and the exon of FMR-1 are indicated. Restriction sites in bold type are sensitive to methylated cytosine residues in CpG dinucleotides. Cen refers to the centromere and tel to the telomere portion of each chromosome. (From Nelson DL. *Fragile X syndrome: Review and Current Status, Growth Genetics & Hormones* 1–4, 1993; with permission.)

protein will include a string of glutamines. Polyglutamine regions have a high charge density, and thus may change the protein’s function and seriously alter cellular operations. If the triplet repeat is outside the coding region in an untranslated region, the ultimate effect may be on mRNA function or gene processing. Table 1 lists several triplet repeat diseases, the identity of the triplets involved, the location of the triplets within the gene, and the theorized result (gain or loss of function) [3]. Although all these diseases are interesting and merit consideration, this discussion will focus primarily on fragile X syndrome, myotonic dystrophy, and Huntingtons disease.

**Fragile X syndrome (Martin-Bell Syndrome)**

*Background*

Fragile X syndrome is the second most common form of genetic mental retardation (after Down syndrome), and is the most common form of familial mental retardation. It accounts for 4% to 8% of all mental retardation in males and females, and is found in all ethnic and racial groups. Affected individuals have a variety of neurologic problems, including mild to severe mental retardation, autistic behavior, attention deficit-hyperactivity disorder, speech and lan-

Table 1  
Comparison of the features of the most common trinucleotide repeat diseases

Disease	Chromosome	Locus	Location in Associated Gene	Repeat	Size in Normal	Size in Carrier	Size in Affected	Change in Gene Function
Kennedy disease (SBMA <sup>a</sup> )	Xq11-12	AR	Exon 1	CAG (gln)	12–34	–	40–62	Gain
Huntington disease	4p16.3	HD	Exon 1	CAG (gln)	6–37	–	35–121	Gain
Spinocerebellar ataxia type 1	6p22-23	SCA1	Exon 8	CAG (gln)	6–39	–	41–81	Gain
Dentatorubral pallidolusyan atrophy	12p12-13	DRPLA	Exon 5	CAG (gln)	7–34	–	54–70	Gain
Machado-Joseph disease	14q32.1	MJD	Internal exon?	CAG (gln)	13–36	–	68–79	Gain
Fragile X syndrome	Xq27.3	FRAXA (FMR1)	5' untranslated	CGG	5–52	43–200	230→>2,000	Loss
Dystrophia myotonica	19q13.3	DM	3' untranslated	CTG (CAG)	5–37	44.46	50→>2,000	RNA stability?
Mental retardation?	Xq27.3	FRAXE	??	GGC (CGG)	6–25	116–133	200→>850	??
(None)	Xq28	FRAXF	??	GGC (CGG)	6–29	–	300–500	??
(None)	16p13.11	FRA16A	??	GGC (CGG)	16–49	–	1,000–2,000	??

From Nelson DL. Allelic expansion underlies many genetic diseases. *Growth Genetics & Hormones* 1996;12:1–4 with permission.

<sup>a</sup> Spinal and bulbar muscular atrophy

guage problems, and occasionally seizures [4]. The physical phenotype includes a narrow face with large jaw, long prominent ears, and macro-orchidism in postpubertal males.

Familial mental retardation affecting only males has been recognized for many years, and in the past was generally classified as *X-Linked mental retardation*. However, the term X-linked is nonspecific, and this generic designation likely included a variety of different X-linked clinical entities. Then in 1969, Lubs described a subgroup of mentally retarded males who had a fragile site in their X chromosome [5]. A fragile site is a specific, non-random point on a chromosome that appears as a nonstaining gap after exposure to certain chemical agents or specific culture conditions [6]. In this case, it was a fragile site at Xq27.3 which became apparent after culturing the cells in folate deficient medium. When this fragile site was also found in the mentally retarded males of a family originally described by Martin and Bell, the Martin-Bell syndrome of X-linked mental retardation became synonymous with fragile X syndrome. Since that original report, four more fragile sites in this area have been discovered. By convention, the original site is called FRAXA and the others are designated FRAXB through E. Only FRAXA and E are associated with (different) mental retardation syndromes.

One of the earliest observations about fragile X syndrome was that it has an unusual inheritance pattern. Although males are primarily affected, a proportion of females are affected as well, and can exhibit a wide range of phenotypic features from very mild to severe. In addition, in contrast to typical X-linked disorders in which only one or a few individuals in every generation is affected, the number of family members with fragile X syndrome typically increases with each generation. This observation came to be called the *Sherman Paradox* after the investigator who first noted that the probability of mental retardation is increased by the number of generations through which the mutation is passed [7]. Most importantly, as the fragile X gene was traced through each family, it became evident that only individuals who inherited the gene from their mothers were affected. Thus, fragile X does not behave like a typical X-linked disorder.

### *Molecular genetics*

The FRAXA site is now known to be a region of unstable DNA within the familial mental retardation (FMR1) gene on the long arm of the X chromosome [8–10]. This unstable region is a series of CGG (cytosine-guanine-guanine) triplet repeats, located in the 5' untranslated region of exon 1, approximately 250 basepairs downstream of a CpG island within the promoter region of the FMR1 gene. Promoter region CpG islands have an important role in the epigenetic control of gene function; they can be methylated, and such methylation acts to stop transcription and effectively turn the gene *off*. It now seems clear that an increased number of CGG triplets in the promoter region of the FMR1 gene somehow triggers CpG methylation and effectively stops transcription of that gene. Most normal individuals have about 29 CGG triplet repeats in the FMR1

promoter region, but it can accommodate up to 55 repeats without any effect on gene function. When the number of repeats is less than 55, methylation and gene silencing do not occur. In addition, a repeat size of 55 or less appears to be fairly stable; expansion from < 55 repeats directly to a full mutation with >200 repeats has never been reported. However, if the number of triplet repeats exceeds 55, the region is unstable. Individuals who have 56 to 199 triplets in this area are said to have a fragile X *premutation*, which can further increase in size as it is transmitted, but only if it is passed from mother to child. If the size of this region reaches  $\geq 200$  repeats (the critical level, corresponding to a full mutation), methylation of the promoter region CpG dinucleotides occurs, and the gene is turned off [11,12]. The loss of gene function leading to loss of the FMR1 protein results in the fragile X phenotype [13]. Thus, both an increased number of CGG repeats and the presence of methylation of the FMR1 gene determine whether an individual is affected [14]. The fact that both gene expansion and methylation must occur before an affected individual exhibits the fragile X phenotype is illustrated by two interesting clinical situations: males carrying the full but unmethylated mutation are phenotypically normal [15], and individuals carrying a smaller but methylated gene are abnormal.

Although it is highly conserved in all species, the function of the FMR1 protein is currently unknown. The FMR1 gene codes for a 4.8 kb mRNA directing the production of a 70 to 80 Kd binding protein that is most active in brain and testes, but also found in placenta, uterus, lung, and kidney [16]. Because the FMR protein binds to mRNA, it may have a regulatory role [17]. As described above, the fragile X phenotype results from a loss of this protein, not from the production of an abnormal protein. Thus, the full fragile X phenotype can also be caused by intragenic loss-of-function mutations, which can range from deletions of the entire gene to loss of only a few kb at the promoter region [18]. The premutation is not associated with any change in FMR1 production, or any of the typical phenotypic features of the fragile X phenotype. However, premutation carriers are at 3- to 4-fold increased risk to develop premature ovarian failure and early menopause (before age 40) [19–21].

### *Prevalence*

The reported carrier rate for fragile X mutations (premutations and full mutations) varies from population to population, ranging from 1/163 to 1/1538 [22]. This wide range of prevalence reflects the influence of a number of variables. For example, the laboratory method used for population testing can impact on results. Southern blot is probably the most accurate testing method, but is not easily adapted for screening large populations. On the other hand, the polymerase chain reaction is best for testing large numbers of samples, but may not be sensitive enough to detect all full and premutations and all mosaics. The number of individuals tested exerts an influence on results, with the most widely disparate estimates of prevalence coming from the smallest studies. Finally, the ethnic or racial background of the tested subjects has a major influence on results. For

example, Rousseau and colleagues found a fragile X prevalence of 1/259 among women in Quebec, but acknowledged that this high prevalence may be due to a founder effect in that the Quebec population descended from a very limited number of settlers [22]. Considering all variables, the incidence of the full fragile X syndrome is generally quoted as 1 per 1000 males and 1 per 2000 females [22,23].

### *Mechanism of gene expansion*

Relatively recent genetic research has clarified and explained some of the interesting features of fragile X transmission. The first question to be answered was, how and why does the number of CGG repeats increase? The exact mechanism is still unknown, but data suggest that the inciting event may be loss of one or more AGG anchors. AGG triplets are usually scattered throughout regions of CGG triplets; in a typical region of 30 CGG repeats, AGG triplets are located at positions 10 and 20. Their location at these sites serves to break up the series of CGGs, which helps to anchor the replication apparatus. If an AGG is lost, slippage during replication is more likely; because of the long uninterrupted string of CGGs, the replication apparatus slips and mistakenly copies some CGGs more than once [3,24] (Fig. 2). The longer the strand, the more prone to slippage; thus, the number of repeats predicts whether or not slippage resulting in an increase in trinucleotide repeats will occur. With 51 repeats, expansion occurs in only 20% of transmitted genes, while with  $\geq 110$  repeats, expansion occurs in 100% [25] (Table 2). On the other hand, the relationship between repeat size and the chance of expansion during transmission is not absolute. The transmission of a premutation through 7 to 8 generations of a large Swedish kindred has been reported [26].

### *Timing of expansion and methylation*

The most interesting aspect of genetic transmission of fragile X is that carrier mothers can have offspring with the full fragile X syndrome, but carrier fathers cannot. This and other clinical observations suggest that expansion of the trinucleotide repeats occurs only if the gene is transmitted by the mother, and that fragile X genes transmitted by the father generally do not change in size (Table 3). This circumstance prompts the question, when do the two steps necessary to inactivate the gene, namely trinucleotide expansion and gene methylation, actually occur? Are these prefertilization events in the oocyte or

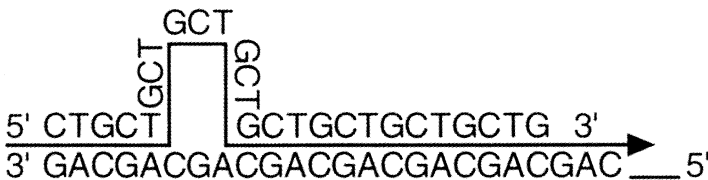


Fig. 2. Diagram showing the presumed mechanism of slippage during replication of genes containing trinucleotide repeats. (From Nelson DL. Allelic expansion underlies many genetic diseases, *Growth Genetics & Hormones* 12:1–4, 1996; with permission.)

Table 2  
Risk of FMR1 gene expansion according to length of trinucleotide repeats

Mutation size	% of offspring with full mutation
50–59	20%
60–67	17%
70–79	39%
80–89	76%
90–99	89%
100–109	91%
110–119	100%
120–129	100%

From Fisch GS, Snow K, Thibodeau SN, et al. The Fragile X premutation in carriers and its effect on mutation size in offspring. *Am J Hum Genet* 1995;56:1147–55 with permission.

sperm, or do they occur only after fertilization? The answer to this question is also currently unknown, but it does seem clear that gene expansion does not occur in the sperm. A variety of evidence suggests that, if anything, the gene may actually contract when transmitted by a male. For example, the sperm of non-mosaic males carrying the full fragile X mutation typically carries only a premutation [27]. Likewise, Reyniers et al. (1993) have reported that mosaic males who carry both premutations and full mutations in their tissues only produce sperm carrying the premutation [28]. It has been hypothesized that sperm carrying the premutation may have a selective advantage over those carrying the full mutation because the smaller FMR1 gene can be replicated faster [29]. Contraction of the full mutation in the fetal testes has also been reported [30].

It is possible that gene expansion occurs in the oocyte. However, there is currently no theory to explain why passage of the trinucleotide repeats through oogenesis could result in expansion. If expansion in the oocyte does occur, genetic imprinting might play a role. The second step, methylation and inactivation of the FMR1 gene, likely occurs after fertilization. This sequence of events is supported by several observations. For example, while fetal and placental tissue usually contain the same size FMR1 gene, the placental tissue is typically hypomethylated while the fetal tissue is methylated. This finding indicates that gene expansion occurred prior to differentiation of the dividing cells into chorionic villus and fetal cells, but that methylation occurred after the division [4].

Many more clinical observations indicate that the expansion likely occurs after fertilization. Somatic cell mosaicism for both the size of the triplet expansion and the degree of methylation, a situation that could only arise in dividing somatic

Table 3  
Parent of origin when gene size changes on transmission-comparison of three triplet repeat diseases

Disease	Size increase	Size decrease	No change in size	Severest phenotype
Fragile X	Mother	[Father, rarely]	Mother or Father	NA
Myotonic Dystrophy	Mother or Father	Father	Mother or Father	Mother
Huntington Chorea	Father	–	Mother	Father

Courtesy Katherine Dowenshrom, MD.

cells, has been reported. Mingroni-Netto and colleagues studied 88 carriers of the fragile X mutation (74 with a premutation and 14 with a full mutation) and their 154 offspring [31]. Fully 9% of the offspring were mosaics, with equal numbers inheriting a smaller, larger, or the same size mutation as their parents. Kruyer and colleagues have described two sets of monozygotic twins carrying the fragile X gene (one male pair, one female) in which the twins were discordant for gene size, methylation status, and phenotype [32]. Because expansion to the full mutation occurred only in their somatic cells and not in their germline cells, it was likely to have occurred after fertilization, during somatic cell mitosis. Thus the fragile X gene in each fetus expanded and became methylated after the zygote split. Furthermore, cases in which mothers carrying the full mutation gave birth to sons who were mosaics for both the premutation and the full mutation [33], and cases in which daughters of fragile X patients inherited only the premutation support the concept of a postzygotic change in the FMR1 gene [34].

### *Genotype-Phenotype Correlation*

The fragile X phenotype varies. Approximately 80% of males and 50 to 70% of females carrying the full mutation are retarded [35–37]. Males are moderately to severely affected, with an IQ in the 35 to 45 range, while the mental retardation in females may be more mild [2]. Twenty percent of males and 10% of females carrying the expanded gene have a very mild phenotype or are unaffected. This phenotypic variability is caused by mosaicism for the size of the expansion, the degree of methylation, or lyonization (in females) [14]. Because mosaicism likely arises during mitosis in the zygote, it cannot be reliably predicted by analysis of either the parental gene or fetal cells, and thus is not amenable to prenatal diagnosis [27,31,38,39]. Women carrying the expanded gene can also have varying degrees of affectation because of lyonization, the random inactivation of one X chromosome in every cell during the late blastocyst stage. Unfavorable lyonization can result in a large proportion or even the majority of cells expressing the expanded fragile X gene [36,40]. The ultimate pattern of lyonization also cannot be predicted prenatally. The factors influencing lyonization and mosaicism (and other aspects of phenotype expression) are not well understood.

### *Diagnosis*

Until recently, the diagnosis of fragile X syndrome was by a cytogenetic technique in which the cells to be tested were cultured in a medium deficient in folate and thymidine. Using this technique, it was possible to identify fragile sites as nonstaining gaps or constrictions on the long arm of the X chromosome (Xq27-28). This technique was unreliable, however, as less than half of cells from affected males manifested this fragile site [14,41,42]. Moreover, this cytogenetic technique was not reliable for carrier testing. For example, Rousseau and colleagues reported that 95% of 278 individuals who carried a premutation were missed by cytogenetic analysis.



The fragile X gene can now be directly examined. The number of CGG repeats and the methylation status of the gene can be determined using Southern blot analysis, because both gene size and the degree of methylation affect the size of the gene fragments obtained after restriction endonuclease digestion. The polymerase chain reaction has been used for testing, but can only determine the number of CGG repeats, not the degree of methylation [4]. Amniocentesis may be preferable to chorionic villus sampling for prenatal diagnosis, because methylation status is difficult to determine in chorionic villus cells, and the methylation pattern in the placenta probably does not reflect methylation in the fetus.

Parents who have a child with mental retardation, developmental delay of unknown etiology, or autism should be encouraged to have their child examined by a geneticist and tested for fragile X using molecular techniques. Approximately 2% to 6% of individuals with these characteristics will be determined to have the fragile X gene expansion [43]. Women who already have a child or other family member with confirmed fragile X syndrome should also be evaluated and counseled by a geneticist; those who are determined to be at risk of having an affected child should be offered prenatal testing. In this situation the patients high-risk status justifies the attempt at fetal diagnosis, even though predicting the phenotype for a fetus who inherits the gene can be difficult.

#### *Obstetric issues-population screening*

Population screening, or testing gravid women or fetuses when there is no family history of fragile X, is controversial. It is not currently recommended by either the American College of Medical Genetics or the American College of Obstetricians and Gynecologists, primarily because prediction of the fetal phenotype, especially when there are no affected family members, is fraught with problems [42,44]. In general, screening for any fetal disease should not be considered unless accurate prenatal diagnosis is available. Currently, accurate phenotype prediction for both male and female fetuses is not always possible, for all the reasons mentioned above.

### **Myotonic dystrophy**

#### *Background*

Myotonic dystrophy is the most common form of adult myopathy. The symptoms range from cataract alone to mild myotonia to severe muscle weakness with pronounced myotonia and mental deterioration. The age of onset varies as well, from birth to 70 years [37]. Interestingly, recognition of genetic anticipation, the phenomenon in which individuals in successive generations of an affected family become symptomatic earlier and to a greater degree than those in the preceding generation, resulted from the study of myotonic dystrophy. Fleisher, a Swiss ophthalmologist, reported in 1918 that patients with

myotonic dystrophy frequently had ancestors with cataracts, and further, that different families with myotonic dystrophy could be linked through mutual ancestors with cataracts [45]. Julia Bell then evaluated these families closely, and showed that affected individuals in each generation after the generation with cataracts had successively more severe disease, occurring earlier in life [46]. However, the concept of genetic anticipation wasn't widely accepted at the time because there was no known genetic mechanism to account for it, and because its main proponent, FW Mott, was an avowed eugenicist, making his scientific colleagues less likely to accept his theories [47]. LS Penrose, a well known and respected geneticist, attributed anticipation to observational biases, pointing out that mildly affected individuals were usually diagnosed only after the birth of a severely affected descendent, but that the reverse (a mildly affected individual is diagnosed first and only then are more severely affected relatives in the preceding generation discovered) rarely occurred [48]. The subsequent recognition of a severe congenital form of the disease, arising when the fetus inherits the gene from an affected mother, supported the concept of anticipation. Then, in 1989, Howeler meticulously evaluated 61 parent-child pairs and showed that the disease virtually always got worse with each generation, not better. In 60 of the 61 pairs studied, the child was affected more severely and earlier than the parent [49].

Although these and other clinical observations revitalized the anticipation theory, the main problem preventing its recognition was that no plausible genetic mechanism by which it could occur was recognized at the time. After 1991, when Fu, Oberle, Verkerk, Yu, and others published studies showing that fragile X was caused by hereditary unstable DNA [9,17,24,50], it was only a matter of time before Buxton and coworkers, Fu and colleagues, and Harley et al. discovered a similar mechanism in myotonic dystrophy [51–54]. The concept of intergenerational triplet repeat expansion, leading to a successively larger and more dysfunctional gene, nicely explained the inheritance pattern. However, subsequent studies have shown that there are some subtle differences between the molecular genetics of fragile X and myotonic dystrophy.

### *Clinical aspects*

Myotonic dystrophy is a multisystem disease characterized by muscle stiffness and progressive dystrophic changes in muscle and in numerous other tissues [55]. Symptoms typically appear for the first time in late childhood or the early adult years, and generally involve distal muscle weakness and atrophy. The facial muscles are then affected, resulting in a classic anhedonic appearance, with temporal wasting, ptosis, and thin neck muscles. The mouth may hang open and dysarthria is common. Muscle disease can be demonstrated by percussing the muscle, which results in sustained muscular contractions, or by electromyography. Eventually the disease affects other organs, causing testicular atrophy, insulin dependant diabetes, gallbladder disease, cardiac arrhythmias, and heart block. Cognitive impairment and cataracts are common.

There are also severe congenital and late adult onset forms of the disease. The development of hydramnios during pregnancy along with decreased fetal movement is a sign that the fetus has severe congenital myotonic dystrophy. At birth such infants are thin and floppy, with facial weakness, diminished cry and suck, and often severe respiratory compromise. As in the adult form of the disease, there is continuous degeneration of affected muscles with limited regeneration, and thus progressive atrophy. Such individuals rarely if ever survive to adulthood. At the other extreme, some gene carriers experience the onset of muscle weakness and atrophy only late in life, or may develop cataracts only. This wide variation in phenotype and the existence of a severe congenital form of myotonic dystrophy is explained by the molecular genetics of the disease.

### *Molecular genetics*

The gene associated with myotonic dystrophy is the myotonin protein-kinase (MT-PK) gene, located on the long arm of chromosome 9 [51,54,56]. This gene has been found to contain a region of CTG trinucleotide repeats, with normal individuals having 3 to 30 repeats and those with myotonic dystrophy having up to 3000 [51,54,56]. This gene has a very low spontaneous new mutation rate [57]; linkage analysis has shown that 58% of British myotonic dystrophy cases and virtually all French Canadian cases are descended from a single ancestor [53].

The triplet repeats are located in an untranslated region of the gene. An expansion in this region leads to a *gain of function* mutation; that is, the triplets result in the production of a new protein, with a new, abnormal function. In this case, the expansion results in the production of an abnormal pre-messenger RNA transcript that inappropriately binds a nuclear ribonucleoprotein called CUG binding protein [58]. This protein binding effectively prevents gene splicing, and prevents the messenger RNA transcript of the expanded gene from leaving the nucleus. The symptoms may be due to reduced levels of normal protein; because the triplet expansion causes haploinsufficiency, only the co-gene produces normal protein, at 50% of the usual amount. On the other hand, rare individuals who are homozygous for the expanded myotonic dystrophy gene do not appear to have more severe symptoms than heterozygotes, suggesting that the symptoms of myotonic dystrophy may be influenced by additional, as yet unknown, factors [59–61]. The clinical effects of the triplet expansion may also be due to nuclear toxicity caused by the trapped mRNA transcripts; toxic damage to the nucleus would be particularly destructive in muscle and nerve cells, which cannot divide [62].

### *Prevalence*

Myotonic dystrophy is the commonest muscular dystrophy of adult life, and has a range of prevalence between 5 and 25 per 100,000 [57,63]. This wide range of prevalence reflects the methods of diagnosis (e.g., were asymptomatic individuals who carry the gene included, were all at-risk relatives tested, etc.). As with fragile X, the founder effect also influences the prevalence of myotonic dystrophy

in certain notable regions of the world, namely in northern Sweden, among South African Afrikaners, and among natives of the Saguenay-Lac St. Jean region of northern Quebec, where the prevalence is exceptionally high. On the other hand, the disease is virtually unheard of in sub-Saharan African populations. The median age at the onset of symptoms is 20 to 25 years in typical myotonic dystrophy, while the severe congenital form is evident at birth or even before.

### *Genotype-phenotype correlation*

There are three distinct myotonic dystrophy phenotypes, which correlate directly with the size of the CTG expansion. This is illustrated by a study by Gennarelli and colleagues, who compared the severity of symptoms to the number of triplet repeats in the DM gene in 465 myotonic dystrophy patients [64]. They found a trimodal distribution in the numbers of triplet repeats, corresponding to three common DM phenotypes. However, as Fig. 3 shows, there was overlap of the number of repeats in all three modes [64]. Individuals with approximately 100 triplet repeats had a 100% probability of having the least severe form of the disease, characterized by minimal signs of myotonia without muscle impairment, mild facial abnormalities (jaw and temporal wasting, facial and sternomastoid weakness, ptosis, nasal speech, frontal balding), cataract, and no distal weakness except isolated flexor weakness of the digits. This phenotype was more common in men (75% men versus 28% women,  $P < 0.001$ ). Individuals with more than 1300 repeats had a 90% chance of having the severest form of the disease, consisting of proximal muscle weakness, cardiomyopathy, endocrine dysfunction, mental retardation, facial abnormalities, and cataract. Those with an intermediate number of repeats, generally between 600 and 800, had an intermediate phenotype, characterized by myotonia, distal weakness, EKG abnormalities, mild mental retardation, gonadal dysfunction, facial abnormalities, and cataract. The intermediate and severe forms of disease appeared to affect men and women equally ( $P = N.S.$ ).

The age at the onset of the disease is also directly correlated with the size of the repeat region; the bigger the repeat region the earlier the onset. Hunter and co-workers studied 109 myotonic dystrophy gene carriers from 17 families, and showed a striking correlation between gene size and age at the onset of symptoms [65]. Individuals shown by linkage analysis to carry the myotonic dystrophy gene, but who had no proven expansion, did not exhibit any symptoms until after age 25, while those with the largest expansion, measuring greater than 4.5 kb, were likely to have the congenital form of the disease. Harley and colleagues studied 439 individuals with myotonic dystrophy clinically and molecularly, and found similar results [66]. Those with adult onset disease generally had a CGT sequence measuring 0.5 to 2.5 kb, those with the childhood form had a sequence measuring 1.5 to 4.0 kb, and in the severe congenital form the sequence typically measured 3.0 to 6.0 kb ( $P < 0.001$ ).

The major area of contrast between myotonic dystrophy and fragile X, however, is the fact that the repeat number can increase during transmission from *either* parent. Furthermore, the number of repeats can also *decrease* when the gene is

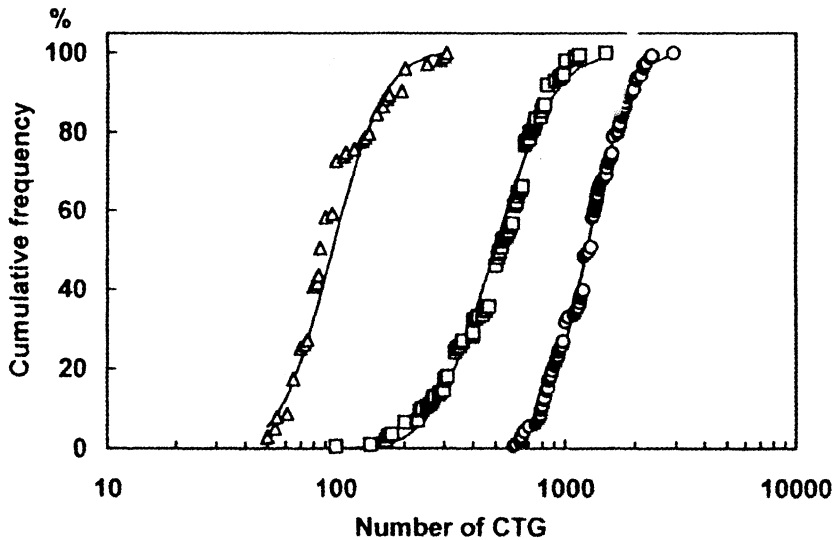


Fig. 3. Trimodal distribution of the length (size) of trinucleotide repeats in myotonic dystrophy patients. Log-normal distribution function of class frequency related to [CTG] repeat number in myotonic dystrophy patients.  $\Delta$ , class 1;  $\square$ , class 2;  $\circ$ , class 3. (From Gennarelli M, Novelli G, Bassi F, et al. Prediction of myotonic dystrophy clinical severity based on the number of intragenic [CTG]<sub>n</sub> trinucleotide repeats. *Am J Med Genet* 65:342–347,1996; with permission.)

transmitted by a male (Table 3). This is illustrated by a large study by Abelovich and colleagues, who evaluated 17 families with 72 members affected with myotonic dystrophy [67]. This series included 15 mothers who transmitted the gene to 23 offspring; in all cases the gene expanded. Eight of these children had congenital myotonic dystrophy, two had the classic form, and four inherited the full mutation but are currently asymptomatic. There were also 15 men who passed the gene on to 30 offspring; in 20 cases the gene expanded, in five the gene decreased in size, and in three the gene size did not change. Seventeen of these children had the classic form, three had mild disease, and seven were asymptomatic carriers. These clinical observations fit with reports of sperm analysis showing a wide range of repeat sizes in the sperm of males with mild myotonic dystrophy [63].

### Diagnosis

Myotonic dystrophy can be reliably diagnosed using molecular methods, which have eliminated the need for muscle biopsy or restriction fragment polymorphism testing of asymptomatic family members. Prediction of the likely ultimate phenotype can usually be done with some accuracy, but the finding of minimally expanded trinucleotide repeats in an asymptomatic but at-risk individual must be interpreted with caution. The issue of whether or not to test asymptomatic but at-risk children is a difficult one, but the general consensus is that, in the absence of symptoms, such testing should be postponed to adult life. In

that way health insurance can be protected and the individual can make his or her own decision about whether or not to be tested.

### *Obstetric issues*

Although men with the disease may be infertile as the result of testicular atrophy, a similar process has not been demonstrated in women. Females with myotonic dystrophy may have menstrual irregularities, but pregnancy can occur. Overall, the fertility of women with myotonic dystrophy is reduced to 2/3 normal levels [63]. However, individually, the effects of the disease are very variable; those with the congenital form usually do not survive to reproductive age, while those with late onset disease may have completed their families before being diagnosed. Women with myotonic dystrophy do seem to have an increased risk of spontaneous pregnancy loss, distinct from losses due to the congenital disease, and ongoing pregnancies are problematic because of prolonged labor, a uterus unresponsive to oxytocin, and uterine atony [68]. More importantly, respiratory compromise can occur after exposure to even small doses of analgesics or anesthetics. Box 1 lists the medications contraindicated in myotonic dystrophy. Copies of this list should be affixed to the hospital charts of myotonic dystrophy patients to avoid inadvertant administration of a potentially toxic drug.

#### **Box 1. Medications believed to be neurotoxic in patients with myotonic dystrophy**

Antibiotics	Neomycin, Lincomycin Tetracycline Polymyxin Gentamycin, streptomycin, kanamycin Penicillamine Colistin
Anesthetics	Procaine, xylocaïne Chloroprocaine, tetracaine Ether Chloroform Trichloroethylene
Analgesics	Morphine sulfate, other narcotics Meperidine Barbiturates
Cardiac medications	Propranolol, other $\beta$ blockers Quinidine $\beta$ adrenergic agents
Miscellaneous	Magnesium sulfate Lithium Quinocrine

## Huntington disease

### *Background*

Huntington disease is an autosomal dominant disorder characterized by progressive chorea, bradykinesia, and rigidity affecting both voluntary and involuntary movements, along with an insidious and slow personality change and deterioration of intellectual function. Depression is common, especially in the early stages of the disease, and is often associated with suicidal ideation. Although the diagnosis of Huntingtons disease has been made as early as two years of age and as late as 86 years, the age at the onset of symptoms is usually 32 to 42 years ( $\pm 10$  years) and the age at death is 50 to 56 years [69]. Approximately 6% of cases have the juvenile form, in which symptoms occur before age 20, and in 25% no symptoms appear until after age 50 [69]. Similar to myotonic dystrophy, the juvenile onset cases are more severe, while the late onset cases are usually characterized by milder symptoms.

### *Molecular genetics*

The Huntington gene (called IT15) has been identified on chromosome 4, and includes a region of CAG triplet repeats in the 5' coding region of the gene. Expansion of the triplet repeats in this region is associated with disease. Normal individuals have 10 to 32 CAG repeats, while those with the disease have 39 to 121 repeats. Individuals with intermediate length repeats (32 to 39) are usually unaffected or have very late onset disease, but can have affected children. The function of the gene product, the huntingtin protein, is not completely understood, but it is believed to be so crucial for normal development that it is considered a *cell survival gene* [70]. In contrast to fragile X, the CAG expansion results in gain of function, not loss [70,71,80]. Gene deletions and other kinds of mutations that result in loss of the IT15 protein do not result in the symptoms of Huntington disease.

### *Prevalence*

The reported prevalence of Huntington disease varies widely, according to the method of case ascertainment (eg, whether or not the figures include pre-symptomatic carriers or at risk individuals who committed suicide before the disease could be diagnosed, etc) and the heritage and ethnic background of the individuals tested. Like myotonic dystrophy, some reports of areas with high prevalence may have been influenced by a founder affect. In addition, for every symptomatic case identified, it is estimated that there are twice as many presymptomatic gene carriers. Some authors estimate that for every symptomatic carrier, there are another five individuals at 50% risk of having the disease and 11 individuals at 25% risk [69]. Considering these facts, the disease prevalence is estimated to be 10 per 100,000.

### *The basis of neuronal damage*

As in fragile X, the CAG triplets code for the amino acid glutamine, and translation results in the addition of an excessively long polyglutamine string to the native protein. The polyglutamine alters the protein's size and charge, and prevents it from being transported or metabolized appropriately [1]. Specifically, huntingtin protein is normally cleaved by a cysteine protease, which plays an important role in apoptosis (programmed cell death). The long polyglutamine tracts appear to enhance the rate of cleavage by this enzyme, thus leading to inappropriately increased apoptosis [70]. Aggregates of this mutant protein can also form inclusion bodies within the nuclei of neurons, which likely contributes to the neuronal loss and gliosis typical of this disease.

There is also evidence indicating that the neuronal damage could result from abnormally strong binding of the mutant huntingtin protein to huntingtin-associated protein, altering the biochemistry of certain brain regions. The brain regions primarily affected by Huntington disease are the caudate, cortex, and globus pallidus. The huntingtin-associated protein is selectively expressed in the caudate and cortex, where it normally binds only weakly to huntingtin [72]. Abnormally strong protein binding in these regions, due to the altered properties of the IT15 protein caused by the polyglutamine insert, could have pathologic consequences.

The abnormal huntingtin protein also binds glyceraldehyde-3-phosphate dehydrogenase, an essential enzyme for glycolysis; the longer the polyglutamine tract, the greater the inhibiting effect on enzyme function [10]. Another effect of the abnormal protein may thus be to inhibit energy utilization in select areas of the brain. Regardless of the exact mechanism of neuronal damage, it is apparent that the size of the polyglutamine repeat, determined by the number of CAG repeats in the huntingtin gene, determines how many years it takes for toxic neuronal changes to occur, and by extension when symptoms will first appear.

### *Genotype-phenotype correlation*

Thus, like myotonic dystrophy, there is a significant correlation between the number of repeats and the age of onset [73,74]; however, the range of instability is much smaller than in myotonic dystrophy, and the correlation with age at onset seems to be confined to the juvenile form of the disease. In fact, repeat length is believed to explain only 50% of the variance of onset age [75]. This was illustrated by Macmillan and colleagues, who analyzed DNA from 449 patients with Huntington disease, and correlated their molecular findings with disease course [76]. The patients with adult onset disease presented with motor abnormalities (77%) or psychiatric disturbance (23%) at a mean age of  $42 \pm 11$  years, and inherited a mean of 42 copies of the CAG repeat (range 16 to 58). Those with the juvenile onset form had a mean age of onset of  $21 \pm 5$  years, and inherited a mean of 60 copies (range 52 to 67). Thus, the age of onset varied over a range of 20 years in the adult onset group, while the number of repeats varied over a range of only 42 copies, much less than in myotonic dystrophy.



There is also a strong relationship between the number of repeats and the type and severity of symptoms in Huntington disease. Many Huntington patients with juvenile onset have a form of the disease called the Westphal variant, a very severe version of the disease characterized by rigidity and akinesia, dystonia, and severe intellectual decline. Affected individuals also frequently have seizures and myoclonus. In contrast, late onset patients typically have very mild symptoms, such as mildly progressive chorea, normal intellect, normal eye movements, and little obvious intellectual or psychiatric change. In fact, in late onset disease, brain pathology may be missed on postmortem examination unless specifically searched for.

The most interesting aspect of disease transmission, which is quite different from the situation in both myotonic dystrophy and fragile X, is that there is a strong correlation between *paternal* inheritance and the early form of the disease [69] (Table 3). Ninety percent of juvenile cases have unusually long CAG repeats and inherit the gene from their father. Several studies regarding the difference in phenotype resulting from maternal versus paternal disease transmission have been published. For example, Ranen and coworkers examined 277 parent-child pairs with Huntington disease [75]. The age at onset of symptoms and the number of triplet repeats in the IT15 gene were known in 60 pairs. These patients were culled from an epidemiologic survey, and thus represented the Huntington disease population fairly accurately. There was no difference in the age at symptom onset between affected mothers and fathers. Likewise, affected mothers and their offspring (male and female) had symptom onset at similar ages; approximately half of the offspring of affected mothers were affected a few years later and half affected a few years earlier than their mothers. However, the offspring of affected fathers had a significantly earlier onset than either their fathers or the offspring of affected mothers. Forty five percent were affected <6 years earlier, 20% were affected between 6 and 12 years earlier, and 35% were affected more than 12 years earlier. Furthermore, 15% of the offspring of affected fathers had the juvenile onset form of the disease compared to only 5% of the offspring of affected mothers, and 77% of the juvenile onset cases had affected fathers. The repeat length correlated with these observations: there was no significant difference in repeat length between affected mothers and their offspring, while the offspring of affected fathers had significantly longer repeat lengths.

The repeat length has been shown to expand during spermatogenesis [71]. It is currently believed that, in direct contrast to fragile X, CAG instability in the huntingtin gene is greater in successive meioses in spermatogenesis than in oogenesis, although the mechanism for this is unclear [69,77].

### *Diagnosis*

The advent of molecular genetic diagnosis for Huntington disease has made it possible both to confirm the diagnosis in symptomatic individuals and to offer pre-symptomatic testing to individuals at risk of inheriting the disease. The region of triplet expansion can be identified and quantified molecularly, and the

relationship between expansion size and symptoms makes it possible to predict the degree of affection within a certain range of accuracy. Because Huntingtons is usually an adult onset disease, many at risk individuals consider presymptomatic testing because they are concerned about their future health status and their reproductive risks. In contrast to fragile X, in which the disease features are evident early in life, and myotonic dystrophy, whose symptoms are primarily muscular, Huntington disease is uniquely terrifying because it strikes otherwise normal adults and involves an insidious neuropsychiatric decline. Most presymptomatic testing programs, in place since the 1980s, have required the patient to undergo extensive psychological counseling before decisions about testing are made because of concerns about possible catastrophic reactions to the test results [78]. Several studies affirming the benefit of such pretest counseling have been performed. For example, Wiggins and colleagues prospectively followed 135 individuals undergoing extensive counseling and presymptomatic Huntington disease testing [78]. They found that, while those who were determined to be at high risk of developing Huntington disease did not experience the same psychological benefit as those receiving more reassuring news, the counseling appeared to have been effective in reducing their level of depression and increasing their sense of well being. Because of the intricacies of testing and test result interpretation, and especially because of the psychological ramifications of the testing process, testing for Huntington disease should be performed in a tertiary center with special expertise in the diagnosis of this disease.

### *Obstetric issues—prenatal diagnosis*

Adult onset Huntington disease usually manifests after the reproductive years, and the juvenile form of the disease is so severe that it is rarely associated with reproduction. The main issue for obstetricians to confront is therefore prenatal diagnosis. Molecular diagnosis has also made it possible to perform prenatal testing. Often, the parent at risk to have the gene and pass it on has not yet been tested, and so should be referred for specialized counseling and consideration of presymptomatic testing before prenatal diagnosis is considered. Once risk has been established, further counseling regarding the ramifications of prenatal testing must be provided. Some authorities are not in favor of prenatal testing for an adult onset disease, especially one in which the precise age at onset and the exact nature of the symptoms cannot be predicted with certainty. In addition, the individual to be tested would likely not be symptomatic for at least 20 years, by which time major advances in therapy may have been made. Furthermore, if the fetus is found to be at risk but the pregnancy is not terminated, the child's health insurance may be jeopardized, and other forms of discrimination may ensue. Having said this, however, prenatal testing for Huntingtons followed by pregnancy termination because of a positive result has been reported [79]. Because of the issues involved, prenatal diagnosis should only be performed in a tertiary center with special expertise in prenatal genetics.

## Other triplet diseases

It is now apparent that triplet repeat expansion is responsible for a number of genetic conditions, primarily neurologic diseases [80]. The list includes Friedrich's ataxia, X-linked spinal and bulbar muscular atrophy (Kennedy's disease), spinocerebellar ataxia types 1 and 2, dentato-rubro-pallido-luysian atrophy, and Machado-Joseph disease. Most of these diseases are associated with gain of function mutations, presumably leading to neural tissue toxicity.

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